REMARKS/ARGUMENTS

By the present amendment, claims 8, 12, 20 and 41 have been amended and claims 45-47 have been deleted rendering claims 1, 4-10, 12-16, 18-20, 24-30, 41, 43 and 44 pending in the application. The amendments to the clams have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further continuation, continuation-in-part or divisional application. The amendment does not contain new matter and its entry is respectfully requested.

Claim Objections

Claim 12 has been amended as requested by the Examiner.

35 USC §112, Second Paragraph

The Examiner has objected to claim 8 under 35 USC §112, second paragraph. In response, claim 8 has been amended in order to replace the article "A" with "The" as requested by the Examiner in the last office action.

In view of the foregoing, we respectfully request that the objection to claim 8 under 35 USC §112, second paragraph be withdrawn.

35 USC §102

The Examiner has rejected claims 20, 25, 26, 28-30, 41, 43 and 44 under 35 USC §102(b) as being anticipated by Hiramatsu et al. (*Appl Environ Microbiol* 56:2125-2132, 1990).

By the present amendment, independent claims 20 and 41 have been amended in order to specify that the full length chymosin pro-peptide is present in the chimeric nucleic acid sequence. As the Examiner has stated, Hiramatsu et al. teaches a vector, JGH2, that contains only five amino acids of the chymosin pro-peptide. Consequently, the claims as amended herewith are not anticipated by Hiramatsu et al.

In view of the foregoing, we respectfully request that the objection to the claims under 35 USC §102(b) be withdrawn.

35 USC §103

The Examiner has rejected claims 1, 4, 6-10, 13-16 and 19 under 35 USC §103(a) as being unpatentable over Hiramatsu et al. (1990) in view of Hiramatsu et al. (*J Biol Chem* 264:16862-16866, 1989). We respectfully disagree with the Examiner for the reasons that follow.

The present invention relates to improved methods and compositions for preparing recombinant proteins in host cells. Prior to the present invention, it was determined that it was desirable to express recombinant proteins as a fusion protein in order to overcome a number of problems. One problem was that overproduced polypeptides can aggregate in the host cell in insoluble fractions known as inclusion bodies. The conversion of this insoluble material involves often slow and complex refolding methods, making protein purification difficult. Another problem was that proteins which are present in soluble form in the cytoplasm often are subject to degradation by host specific enzymes, thus reducing the amounts of active protein that can be recovered. Linking the polypeptide of interest to a fusion partner has been found to limit these problems. However, in order to recover the active polypeptide it is generally necessary to separate the fusion partner from the polypeptide of interest. Currently this is achieved through either an enzymatic means (e.g. by the addition of proteolytic enzymes) or chemical means (e.g. the addition of cyanogenbromide). Chemical cleavage often requires elevated temperature and toxic compounds which denature the recombinant protein and complicate purification. The prior art cleavage methods have been recognized to be either inefficient or lack cleavage specificity.

The present invention overcomes the problems of the prior art by providing compositions and methods for the **inexpensive, readily available** and **efficient** cleavage of recombinant fusion proteins. The method of the pending claims involves preparing the desired recombinant protein as a fusion protein with a chymosin pro-

peptide sequence which is located immediately upstream of the recombinant protein without the mature form of the chymosin intervening. Upon the addition of a mature aspartic protease, the recombinant protein is efficiently cleaved from the pro-peptide. The novelty of the claimed invention is supported by the publication of the technology in a peer-reviewed journal, Protein Engineering, a copy of which we enclose. None of the prior art cited by the Examiner, either alone or in combination, would lead one of skill in the art to the present invention.

Hiramatsu et al. (1990) teaches only one vector JGH2 that contains part of a pro-sequence of Mucor Pusillus Rennin (MPR). The JGH2 vector comprises the full length pre-sequence and only five amino acids of the pro-sequence fused to human growth hormone via a 3 amino acid linker sequence. Importantly, Hiramatsu (1990) reports that they did not get efficient cleavage of the 5 amino acids of the pro-sequence from the hGH sequence with only part of the pro-sequence. In particular, they found that cleavage had occurred at the junction of the pre- and pro-sequences thus yielding an hGH sequence with 5 amino acids of the pro-sequence still N-terminally attached thereto. Therefore, Hiramatsu et al. (1990) did not achieve the present invention wherein the pro-peptide sequence is completely cleaved from the recombinant polypeptide. In this regard, we direct the Examiner to part (c) of claim 1 wherein it is stated that "the chymosin pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide".

Hiramatsu et al. (1990) realized that it is undesirable to have part of the pro-peptide linked to the desired recombinant protein and expressly stated that:

> "Although **removal of the extra amino acids of the pro-peptide is required** to obtain hGH with the same NH_2-terminus as native hGH, it may be achieved by the **introduction of an artificial process site** ... just before the hGH sequence" (emphasis ours).

The Examiner states that the introduction of a cleavage site is only a suggestion by Hiramatsu et al. We strongly emphasize that it is the only suggestion offered by

Hiramatsu and the only solution tried by Hiramatsu. In this regard, in their later publication, (Hiramatsu et al., *Applied and Environmental Microbiol*, Vol. 57, No. 7, p. 2052-2056, July 1991), Hiramatsu introduced an artificial linker that is recognized by the yeast KEX2 protease into their vectors in order to get efficient cleavage.

Therefore, while Hiramatsu et al. recognized the problem in the prior art, they provide no motivation to one of skill in the art to solve the problem by the method of the present invention. One of skill in the art having read Hiramatsu's work would in no way be lead to solve the problem by the method of the present invention. We respectfully submit that if the solution taught by the present invention was "obvious", then it at least would have been suggested in one of Hiramatsu's publications as they were clearly looking for a solution to the problem. It is important to note that Hiramatsu et al. does not add a mature form of an aspartic protease to effect cleavage of the pro-peptide as is specified in step (c) of pending claim 1.

The deficiencies in Hiramatsu et al. (1990) are clearly not remedied by Hiramatsu et al. (1989) as Hiramatsu et al. (1989) is merely describing the expression and cleavage of wild-type pre-pro chymosin. The fact that the pro-sequence is efficiently cleaved from the chymosin is not unexpected as that is how zymogens function. However, Hiramatsu et al. (1989) would provide no motivation or suggestion to one of skill in the art to use the pro-peptide sequence of chymosin for the efficient expression and recovery of a recombinant protein. This is again evidenced by the later Hiramatsu et al. publications (1990 and 1991) wherein they expressed a need for an artificial cleavage site and then go on to prepare such a construct without suggesting any alternate solutions.

The Examiner has rejected claim 5 under 35 USC §103(a) as being unpatentable over Hiramatsu et al. (1990) in view of Hiramatsu et al. (1989) as applied to claims 1, 4, 6-10, 13-16 and 19 above and further in view of Fine et al. (*Gen Comp Endocrinol* 89:51-61) and the rejection of claim 24 as being unpatentable over Hiramatsu et al. (1990) in view of Fine et al. We respectfully disagree with the Examiner for the reasons that follow.

Claims 5 and 24 relate to specific embodiments of the invention wherein the heterologous protein is carp growth hormone. As a result, these claims carry with them all of the novel and inventive features of claims 1 and 20 from which these claims depend. Our comments on the Hiramatsu et al. references appear above and equally apply to these claims. The deficiencies in the Hiramatsu et al. references are in no way remedied by Fine which is a reference that describes expression of Carp Growth Hormone (cGH) in *E.coli*, purification of cGH from *E.coli*, *in vitro* (using lymphoma and preadipocyte cells) and *in vivo* (evaluating growth rate in fish injected with the purified protein) characterization of cGH. Fine in no way teaches or suggests an improved method to prepare cGH by linking the cGH to a pro-peptide from chymosin.

We remind the Examiner that he is to consider secondary considerations when assessing obviousness. Secondary considerations relevant to the present invention include long-felt need in the art. The expression of valuable recombinant proteins (such as therapeutic proteins) is highly desirable. However, as stated previously, there are difficulties recognized the art with respect to the efficient production and recovery of recombinant proteins. The present invention solves the difficulties of the prior art by providing an efficient method of cleaving recombinant proteins. Further, the present invention permits the use of fusion proteins for larger scale manufacturing, both by reducing the cost of the cleavage agent and by addressing safety issues arising from the use of certain cleavage agents. Therefore, there is a clear long felt need in the art for the present invention which must be given due weight when considering inventive step.

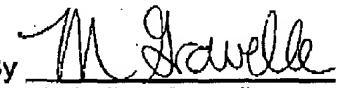
In view of the foregoing, we respectfully request that all of the objections under 35 USC §103 be withdrawn.

The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner like to discuss the matter, he is kindly requested to contact Micheline Gravelle at 416-957-1682 at his convenience.

Respectfully submitted,

BERESKIN & PARR

By 
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Attachments